

Figure 6: Synthetic ODN cannot be mixed with DNA vaccine due to interference with expression from plasmid. The figure shows the effect of adding S-ODN to plasmid DNA expressing reporter gene or antigen. ODN 1826 (10 or 100 µg) was added to DNA constructs (10 µg) encoding hepatitis B surface antigen (HBsAg) (pCMV-S, top panel) or luciferase (pCMV-luc, bottom panel) DNA prior to intramuscular (IM) injection into mice. There was an ODN dose-dependent reduction in the induction of antibodies against HBsAg (anti-HBs, end-point dilution titers at 4 wk) by the pCMV-S DNA (top panel) and in the amount of luciferase expressed in relative light units per sec per mg protein (RLU/sec/mg protein at 3 days) from the pCMV-luc DNA (bottom panel). This suggests that the lower humoral response with DNA vaccine plus ODN was due to decreased antigen expression. Each bar represents the mean of values derived from 10 animals (top panel) or 10 muscles (bottom panel) and vertical lines represent the SEM. Numbers below the bars indicate proportion of animals responding to the DNA vaccine (top panel); all muscles injected with pCMV-luc expressed luciferase (bottom panel).

Please re-write the paragraph beginning on page 6, line 13, as follows:

Figure 7: Interference of ODN with pDNA due to backbone and sequence. The figure shows the interference of ODN with plasmid DNA depends on backbone and sequence. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 days after they were injected with 10 µg pCMV-luc DNA to which had been added no ODN (none = white bar) or 100 µg of an ODN, which had one of three backbones: phosphorothioate (S = left slanted bars: 1628, 1826, 1911, 1982, 2001 and 2017), phosphodiester (O = thick left slanted bar: 2061), or a phosphorothioate-phosphodiester chimera (SOS = right slanted bars: 1585, 1844, 1972, 1980, 1981, 2018, 2021, 2022, 2023 and 2042). Three S-ODN (1911, 1982 and 2017) and two SOS-ODN (1972 and 2042) did not contain any immunostimulatory CpG motifs. One S-ODN (1628) and three SOS-ODN (1585, 1972, 1981) had poly-G ends and one SOS-ODN (2042) had a poly-G center. The (*) indicates ODN of identical sequence but different backbone: 1826 (S-ODN), 1980 (SOS-ODN) and 2061 (O-ODN). All S-ODN (both CpG and non-CpG) resulted in decreased luciferase activity whereas SOS-ODN did not unless they had poly-G sequences.

Please re-write the paragraph beginning on page 6, line 25, as follows:

Figure 8: Temporal and spatial separation of CpG ODN and plasmid DNA. The figure shows the effect of temporal or spatial separation of plasmid DNA and S-ODN on gene

expression. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 or 14 days after they were injected with 10 µg pCMV-luc DNA. Some animals also received 10 µg CpG-S ODN which was mixed with the DNA vaccine or was given at the same time but at a different site, or was given 4 days prior to or 7 days after the DNA vaccine. Only when the ODN was mixed directly with the DNA vaccine did it interfere with gene expression.

Please re-write the paragraph beginning on page 7, line 6, as follows:

Figure 9: Immunization of BALB/c mice with CpG-optimized DNA vaccines. The figure shows the enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 µg of pUK-S, pMAS-S, pMCG16-S or pMCG50-S plasmid DNA bilaterally (50 µl at 0.1 mg/ml in saline) into the TA muscle. The top panel shows the anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means (n=5) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. The bottom panel shows the cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means (n=3) for % specific lysis (performed in triplicate) at an effector:target (E:T) ratio of 10:1, dots represent the individual values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 7, line 19, as follows:

Figure 10 shows induction of a Th2-like response by a CpG-N motif and inhibition of the Th1-like response induced by a CpG-S motif. Anti-HBs antibody titers (IgG1 and IgG2a subclasses) in BALB/c mice 12 weeks after IM immunization with recombinant HBsAg, which was given alone (none) or with 10 µg stimulatory ODN (1826), 10 µg of neutralizing ODN (1631, CGCGCGCGCGCGCGCGCGCG (SEQ ID NO:22); 1984, TCCATGCCCGTTCCTGCCGTT (SEQ ID NO:78); or 2010 GCGGCGGGCGGCGCGCGCCC (SEQ ID NO:75); CpG dinucleotides are underlined for clarity) or with 10 µg stimulatory ODN + 10 µg neutralizing ODN. To improve nuclease resistance for these *in vivo* experiments, all ODN were phosphorothioate-modified. Each bar represents the group mean (n=10 for none; n=15 for #1826 and n=5 for all other groups) for anti-HBs antibody titers as determined by end-point dilution ELISA assay. Hatched portions

of bars indicate antibodies of IgG1 subclass (Th2-like) and white portions indicate IgG2a subclass (Th1-like). The numbers above each bar indicate the IgG2a/IgG1 ratio where a ratio >1 indicates a predominantly Th1-like response and a ratio <1 indicates a predominantly Th2-like response (a value of 0 indicates a complete absence of IgG2a antibodies).

Please re-write paragraph beginning on page 8, line 5, as follows:

Figure 11 shows enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 μ g of pUK-S (white bars), pMAS-S (right slanted bars), pMCG16-S (thin right slanted bars) or pMCG50-S (left slanted bars) plasmid DNA bilaterally (50 μ l at 0.1 mg/ml in saline) into the TA muscle. Panel A: The anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means ($n=5$) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. Panel B: Cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means ($n=3$) for % specific lysis (performed in triplicate) at an effector: target (E:T) ratio of 10:1, dots represent the individual values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 35, line 8, as follows:

(i) Insertion of the CMV (human cytomegalovirus) major intermediate early promoter/enhancer region

The CMV promoter (from pcDNA3 position 209 to 863) was amplified by PCR using 30 ng pcDNA3 as a template. The forward PCR primer 5'CGT GGA TAT CCG ATG TAC GGG CCA GAT AT 3'(SEQ ID NO:4) introduced an EcoRV site, and the reverse PCR primer 5' AGT CGC GGC CGC AAT TTC GAT AAG CCA GTA AG 3'(SEQ ID NO:5) introduced a *NotI* site. After digestion with EcoRV and *NotI*, a 0.7 kb PCR fragment containing the CMV promoter was purified and inserted into the pUK21 polylinker between *XbaI* and *NotI* sites. The *XbaI* sticky end of pUK21 was filled in with the large fragment of T4 DNA polymerase after digestion to create a blunt end. The inserted CMV promoter was confirmed by sequencing. The resulting plasmid was pUK21-A1 (Figures 1A and 1B).

Please re-write the paragraph beginning on page 35, line 19, as follows:

(ii) Insertion of the BGH polyA (bovine growth hormone polyadenylation signal)

BGH polyA (from pcDNA3 position 1018 to 1249) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' ATT CTC GAG TCT AGA CTA GAG CTC GCT GAT CAG CC 3' (SEQ ID NO:6) introduced *XhoI* and *XbaI* sites, and the reverse PCR primer 5' ATT AGG CCT TCC CCA GCA TGC CTG CTA TT 3' (SEQ ID NO:7) introduced a *StuI* site. After digestion with *XhoI* and *StuI*, the 0.2 kb PCR fragment containing the BGH polyA was purified, and ligated with the 3.7 kb *XhoI-StuI* fragment of pUK21-A1. The inserted BGH polyA was confirmed by sequencing. The resulting plasmid was pUK21-A2 (Figures 2A and 2B).

Please re-write the paragraph beginning on page 36, line 24, as follows:

(i) Insertion of the fl origin of replication region

The fl origin and two unique restriction enzyme sites (*DraI* and *ApaI*) were introduced into pUK21-A2 for later vector construction. fl origin (from pcDNA3 position 1313 to 1729) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' TAT AGG CCC TAT TTT AAA CGC GCC CTG TAG CGG CGC A 3' (SEQ ID NO:8) introduced *EcoO109I* and *DraI* sites, and the reverse PCR primer 5' CTA TGG CGC CTT GGG CCC AAT TTT TGT TAA ATC AGC TC 3' (SEQ ID NO:9) introduced *NarI* and *ApaI* site. After digestion with *NarI* and *EcoO109I*, the 0.4 kb PCR fragment containing the fl origin was purified and ligated with the 3.3 kb *NarI-EcoO109I* fragment of pUK21-A2, resulting in pUK21-A (Figures 3A and 3B).

Please re-write the paragraph beginning on page 38, line 22, as follows:

(iii) Replacement of the fl origin with unique restriction enzyme sites

Oligonucleotides 5' AAA TTC GAA AGT ACT GGA CCT GTT AAC A 3' (SEQ ID NO:10) and its complementary strand 5' CGT GTT AAC AGG TCC AGT ACT TTC GAA TTT 3' (SEQ ID NO:11) were synthesized, and 5'-phosphorylated. Annealing of these two phosphorylated oligos resulted in 28 base pair double-stranded DNA containing three unique restriction enzyme sites (*ScaI*, *AvaII*, *HpaI*), one sticky end and one blunt end. Replacing the 0.4 kb *NarI-DraI* fragment of pUK21-B with this double-stranded DNA fragment resulted in the universal vector pMAS for DNA vaccine development (Figures 4A and 4B and 5).

Please re-write the paragraph beginning on page 44, line 11, as follows:

In contrast to the success with protein antigens, attempts to augment immune responses induced by a HBsAg-expressing DNA vaccine by the addition of CpG-S ODN 1826 failed. Surprisingly, the immune responses decreased with the addition of CpG-S ODN in a dose-dependent manner (Figure 6, top panel). Addition of ODN #1826 to a luciferase reporter gene construct (pCMV-luc, Davis *et al.*, 1993b) resulted in a dose-dependent decrease in luciferase expression (Figure 6, bottom panel). This indicates that the negative effects of the CpG-S ODN on the DNA vaccine were due to reduced gene expression rather than an effect on the immune response against the gene product.

Please re-write the paragraph beginning on page 48, line 15, as follows:

Next, different numbers of CpG-S motifs were inserted into the vector by allowing self-ligation of a 20bp DNA fragment with the sequence 5'

~~GACTCCATGACGGTTCCTGACGTTTCCATGACGTTTCCTGACGTTT~~ 3' (SEQ ID NO:12) with a complementary strand and inserting different numbers of copies into the *Ava*II site of pMAS. Recombinant clones were screened and the two vectors were chosen for further testing with 16 and 50 CpG-S motifs, and named pMCG16 and pMCG50 respectively.

Please re-write the paragraph beginning on page 51, line 16, as follows:

When tested for their ability to induce cytokine (IL-6 and IL-12) secretion from cultured spleen cells, we found that the pMAS-S, pMCG16-S and pMCG50-S vectors had significantly enhanced immune stimulatory activity compared to pUK-S. When used as a DNA vaccine, the anti-HBs response at 4 and 6 weeks was substantially stronger with DNA vaccines from which CpG-N motifs had been deleted, and even more so when 16 CpG-S motifs had been inserted. The vector with 50 CpG-S motifs, however, was less effective at inducing antibody production than that with 16 motifs. (Figure 11, panel A). Removal of CpG-N motifs and addition of CpG-S motifs resulted in a more than three-fold increase in the proportion of IgG2a relative to IgG1 anti-HBs antibodies, indicating an enhanced Th-1 response. This accentuated Th1 response also was demonstrated by the striking progressive increases in CTL responses induced by vectors from which CpG-N motifs were deleted and/or CpG-S motifs added (Figure 11, panel B).

Please re-write the paragraph beginning on page 53, line 20, as follows:

Based on our *in vitro* experiments we hypothesized that the presence of CpG-N motifs in DNA vaccines interferes with the induction of the desired immune response. Indeed, the

present study demonstrates that elimination of CpG-N motifs from a DNA vaccine leads to improved induction of antibodies. By removing 52 of the CpG-N motifs from a DNA vaccine (45 were deleted and 7 turned into CpG-S motifs) the serologic response was more than doubled; by then adding an additional 16 CpG-S motifs, the response was enhanced nearly 10 fold (Figure 11, panel A). Likewise, CTL responses were improved by removing CpG-N motifs and even more so by adding 16 or 50 CpG-S motifs (Figure 11, panel B). These increased responses are especially notable in view of the fact that the total number of CpG dinucleotides in the mutated vaccines is considerably below the original number.

Please re-write the paragraph beginning on page 54, line 2, as follows:

The finding that the vector with 50 CpG-S motifs was inferior to that with 16 motifs for induction of humoral immunity was unexpected, and may be secondary to CpG-induced ~~production of type I interferons, and subsequent reduction in the amount of antigen~~ expressed. The decreased antibody response induced by pMCG50-S seems unlikely to be explained by vector instability since this vector gave the best CTL responses (Figure 11, panel B). Although the pMCG50-S vector was slightly larger than pMCG16-S, the 10 μ g dose still contained 93% as many plasmid copies as it did pMCG16-S, so lower copy number is unlikely to account for the reduced antibody levels. The current generation of DNA vaccines are quite effective in mice, but much less effective in primates (Davis, H.L., *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:7213-7218 (1996); Letvin, N.L., *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:9378-9383 (1997); Fuller, D.H., *et al.*, *J Med. Primatol.*, 25:236-241 (1996); Lu, S., *et al.*, *J Virol.*, 70:3978-3991 (1996); Liu, M.A., *et al.*, *Vaccine*, 15:909-919 (1997); Prince, A.M., *et al.*, *Vaccine*, 15:9196-919 (1997); Gramzinski, R.A., *et al.*, *Molec. Med.*, 4:109-119 (1998)). Our present results indicate that attaining the full clinical potential of DNA vaccines will require using engineered vectors in which CpG-N motifs have been deleted, and CpG-S motifs added.

Please re-write Table 1, beginning on page 56, line 22, as follows:

Table 1.

Primers used for site-directed mutagenesis.

Mutated nucleotides are underlined. Restriction enzyme sites for cloning, are indicated in bold.

Forward primers:

Mu-0F	5' GTCTCTAGACAGCCACTGGTAACAGGATT 3' (845) (SEQ ID NO:23)
Mu-1F	(1144) 5' <u>GTCTTGTGTC</u> GTCAAGTCAGCGTAATGC 3' (1172) (SEQ ID NO:24)
Mu-2F	(1285) 5' <u>TCGTTTCTGTAATGAAGGAG</u> 3' (1304) (SEQ ID NO:25)
Mu-3F	(1315) 5' <u>AAGGCAGTTCCATAGGATGG</u> 3' (1334) (SEQ ID NO:26)
Mu-(4+5)F	(1348) 5' TCGATCTGCGATTCC <u>AACT</u> CGTCCAACATCAATAC 3' (1382) (SEQ ID NO:27)
Mu-6F	(1453) 5' <u>TGGTGAGAATGGCAAAAGTT</u> 3' (1472) (SEQ ID NO:28)
Mu-7F	(1548) 5' CATTATTCATTCGTGATTGCG 3' (1568) (SEQ ID NO:29)
Mu-8F	(1633) 5' <u>ACGTC</u> CAGGAACACTGCCAGCGC 3' (1656) (SEQ ID NO:30)
Mu-9F	(1717) 5' <u>AGGGATCGCAGTGGTGAGTA</u> 3' (1736) (SEQ ID NO:31)
Mu-10F	(1759) 5' <u>TATAAAATGCTTGATGGTCGG</u> 3' (1779) (SEQ ID NO:32)
Mu-(11+12)F	(1777) 5' <u>GGAAGAGGCATAAATTC</u> TGTCAGCCAGTTTAGTC 3' (1811) (SEQ ID NO:33)
Mu-13F	(1882) 5' <u>TGGCTTCCCATACAAGCGAT</u> 3' (1901) (SEQ ID NO:34)
Mu-14F	(1924) 5' <u>TACATTATCGCGAGCCCAT</u> T 3' (1943) (SEQ ID NO:35)
Mu-15F	(1984) 5' <u>TGGCCTCGACGTTTCCCGT</u> 3' (2002) (SEQ ID NO:36)

Reverse primers:

Mu-0R	5' ATCGAATTCAGGGCC <u>TCGTGATACGCCTA</u> 3' (2160) (SEQ ID NO:37)
Mu-1R	(1163) 5' TGA <u>CTTGACGACACAACGAC</u> AGCTCATGACCAAAATCCC 3' (1125) (SEQ ID NO:38)
Mu-2R	(1304) 5' CTCCTTCATTACAGAAACG <u>ACTTTTTCAAAAATATGGTA</u> 3' (1266) (SEQ ID NO:39)
Mu-3R	(1334) 5' CCATCCTATGGA <u>ACTGCC</u> TGGTGAGTTTCTCCTTC 3' (1298) (SEQ ID NO:40)
Mu-(4+5)R	(1367) 5' GAGT <u>TGGAATCGCAG</u> ATCGATACCAGGATCTTGC 3' (1334) (SEQ ID NO:41)
Mu-6R	(1472) 5' AACTTTTGCCATTCTCACC <u>AGATT</u> CAGTCGTCCTCA 3' (1436) (SEQ ID NO:42)
Mu-7R	(1568) 5' CGCAATCACGAATGAATAA <u>TGGTTTGGTTGATGCGAGTG</u> 3' (1530) (SEQ ID NO:43)

Mu-8R (1652) 5' TGGCAGTGTTCCTGAGACGTTTGCATTTCGATTCCCTGTT 3' (1615) (SEQ ID NO:44)

Mu-9R (1736) 5' TACTCACCCTGCGATCCCTGGAAAAACAGCATTCCAG 3' (1736) (SEQ ID NO:45)

Mu-10R (1779) 5' CCGACCATCAAGCATTTTATACGTACTCCTGATGATGCA 3' (1741) (SEQ ID NO:46)

Mu-(11+12) (1796) 5' CAGAATTTATGCCTCTTCCCACCATCAAGCATTTTATAC 3' (1758) (SEQ ID NO:47)

Mu-13R (1901) 5' ATCGCTTGTATGGGAAGCCAGATGCGCCAGAGTTGTTT 3' (1882) (SEQ ID NO:48)

Mu-14R (1943) 5' AATGGGCTCGCGATAATGTAGGGCAATCAGGTGCGAC 3' (1907) (SEQ ID NO:49)

Mu-15R (2002) 5' ACGGGAAACGTCGAGGCCACGATTAAATTCCAACATGG 5' (1965) (SEQ ID NO:50)

T09260 T09260 T09260

Please re-write Table 2, beginning on page 59, line 1, as follows:

Table 2 Nucleotide and amino acid sequences of the *AlwNI-EcoO109I* fragment (SEQ ID NO:80)

kan (wt)	2180	AAGGGCCTCG	TGATACGCCT	ATTTTTATAG	GTTAATGTCA	TGGGGGGGGG	GGGGAAAGCC
kan (wt)	2120	ACGTTGTGTC	TCAAAATCTC	TGATGTTACA	TTGCACAAGA	TAAAAATATA	TCATCATGAA
kan (wt)	2060	CAATAAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC	CATATTCAAC
kan (mu)							
ORF						M S	H I Q
kan (wt)	2000	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATGC	TGATTTATAT	GGGTATAAAT
kan (mu)			A				
ORF		R E T S	R P R	L N S	N M D A	D L Y	G Y K
kan (wt)	1940	GGGCTCGCGA	TAATGTCCGG	CAATCAGGTG	CGACAATCTA	TCGCTTGTAT	GGGAAGCCCG
kan (mu)			A				A
ORF		W A R D	N V G	Q S G	A T I Y	R L Y	G K P
kan (wt)	1880	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT	GTTACAGATG
kan (mu)							
ORF		D A P E	L F L	K H G	K G S V	A N D	V T D
kan (wt)	1820	AGATGGTCAG	ACTAAACTGG	CTGACGGAAT	TTATGCCTCT	TCCGACCATC	AAGCATTTTA
kan (mu)				A		C	
ORF		E M V R	L N W	L T E	F M P L	P T I	K H F
kan (wt)	1760	TCCGTACTCC	TGATGATGCA	TGGTTACTCA	CCACTGCGAT	CCCCGGAAAA	ACAGCATTCC
kan (mu)		A				T	
ORF		I R T P	D D A	W L L	T T A I	P G K	T A F
kan (wt)	1700	AGGTATTAGA	AGAATATCCT	GATTCAAGTG	AAAATATTGT	TGATGCGCTG	GCAGTGTTC
kan (mu)							
ORF		Q V L E	E Y P	D S G	E N I V	D A L	A V F
kan (wt)	1640	TGCGCCGTT	GCATTCGATT	CCTGTTTGTA	ATTGTCCTTT	TAACAGCGAT	CGCGTATTT
kan (mu)		A A A					
ORF		L R R L	H S I	P V C	N C P F	N S D	R V F
kan (wt)	1580	GTCTCGCTCA	GGCGCAATCA	CGAATGAATA	ACGGTTTGGT	TGATGCGAGT	GATTTTGATG
kan (mu)					T		
ORF		R L A Q	A Q S	R M N	N G L V	D A S	D F D
kan (wt)	1520	ACGAGCGTAA	TGGCTGGCCT	GTTGAACAAG	TCTGGAAAGA	AATGCATAAA	CTTTTGCCAT
kan (mu)							
ORF		D E R N	G W P	V E Q	V W K E	M H K	L L P
kan (wt)	1460	TCTCACCGBA	TTCAGTCGTC	ACTCATGGTG	ATTTCTCACT	TGATAACCTT	ATTTTGGACG
kan (mu)		A					
ORF		F S P D	S V V	T H G	D F S L	D N L	I F D
kan (wt)	1400	AGGGGAAATT	AATAGGTTGT	ATTGATGTTG	GACGAGTCGG	AATCGCAGAC	CGATACCAGG
kan (mu)					T	T	
ORF		E G K L	I G C	I D V	G R V G	I A D	R Y Q
kan (wt)	1340	ATCTTGCCAT	CCTATGGAAC	TGCCTCGGTG	AGTTTCTCC	TTCATTACAG	AAACGGCTTT
kan (mu)				T			T
ORF		D L A I	L W N	C L G	E F S P	S L Q	K R L
kan (wt)	1280	TTCAAAAATA	TGGTATTGAT	AATCCTGATA	TGAATAAATT	GCAGTTTCAT	TTGATGCTCG
kan (mu)							
ORF		F Q K Y	G I D	N P D	M N K L	Q F H	L M L
kan (wt)	1220	ATGAGTTTTT	CTAATCAGAA	TTGGTTAATT	GGTTGTAACA	CTGGCAGAGC	ATTACGCTGA
kan (mu)							
ORF		D E F F					
kan (wt)	1160	CTTGACGGGA	CGGCGCAAGC	TCATGACCAA	AATCCCTTAA	CGTGAGTTTT	CGTTCCACTG
kan (mu)		AC	AA AC				
kan (wt)	1100	AGCGTCAGAC	CCCGTAGAAA	AGATCAAAGG	ATCTTCTTGA	GATCCTTTTT	TTCTGCGCGT
kan (wt)	1040	AATCTGCTGC	TTGCAACAA	AAAAACCACC	GCTACCAGCG	GTGGTTTGTT	TGCCGGATCA
kan (wt)	980	AGAGCTACCA	ACTCTTTTTC	CGAAGGTAAC	TGGCTTCAGC	AGAGCGCAGA	TACCAATAC
kan (wt)	920	TGTTCTTCTA	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	CACCGCTAC
kan (wt)	860	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC		

Note: Mutated nucleotides are underlined. The *AlwNI* and *EcoO109I* sites are indicated in bold type. The nucleotide numbering scheme is the same as the backbone vector pUK21.

09965101.092601

Please re-write Table 3, beginning on page 60, line 1, as follows:

Plasmid DNA Vectors

Davis *et al.* (1998)

Table 3

Plasmids containing immunostimulatory CpG motifs

Plasmid	Backbone	No. CpG Motifs	Species Specificity and ODN Equivalence of CpG-S Insert
pMCG-16	pMAS	16	mouse-specific CpG motif #1826 ¹
pMCG-50	pMAS	50	
pMCG-100	pMAS	100	
pMCG-200	pMAS	200	
pHCG-30	pMAS	30	human-specific CpG motif - no ODN equivalent ²
pHCG-50	pMAS	50	
pHCG-100	pMAS	100	
pHCG-200	pMAS	200	
pHIS-40	pMAS	40	human-specific CpG motif #2006 ³
pHIS-64	pMAS	64	
pHIS-128	pMAS	128	
pHIS-192	pMAS	192	

¹ sequence of 1826 is TCCATGACCGTTCTGACCGTT (SEQ ID NO:51)

² sequence used as a source of CpG motifs is
GACTTCGTGTCCGTTCTTCTGTCGTCTTTAGCGCTTCTCCTGCGTGCGTCCCTTG (SEQ ID NO:14)

³ sequence of 2006 is TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:3)

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Please re-write Table 4, beginning on page 61, line 1, as follows:

Table 4

Plasmids encoding hepatitis B surface antigen (derived from ayw or adw subtypes of HBV)

Plasmid	Backbone	Insert
pUK-S	pUK21-A2	HBV-S (ayw)
pUKAX-S	pUK21-AX*	HBV-S (ayw)
pMAS-S	pMAS	HBV-S (ayw)
pMCG16-S	pMCG-16	HBV-S (ayw)
pMCG50-S	pMCG-50	HBV-S (ayw)
pMCG100-S	pMCG-100	HBV-S (ayw)
pMCG200-S	pMCG-200	HBV-S (ayw)
pHCG30-S	pHCG-30	HBV-S (ayw)
pHCG50-S	pHCG-50	HBV-S (ayw)
pHCG100-S	pHCG-100	HBV-S (ayw)
pHCG200-S	pHCG-200	HBV-S (ayw)
pHIS40-S(ad)	pHIS-40	HBV-S (adw2)
pHIS64-S(ad)	pHIS-64	HBV-S (adw2)
pHIS128-S(ad)	pHIS-128	HBV-S (adw2)
pHIS192-S(ad)	pHIS-192	HBV-S (adw2)

*pUK21-AX was created by deleting fl origin from pUK21-A

Please re-write Table 5, beginning on page 62, line 1, as follows:

Table 5 *Sequence comparison of pUK21-A2 (SEQ ID NO:83) and pGT (SEQ ID NO:84). 75 point-mutations (indicated with *) in pUK21-A2 results in the gene therapy vector (pGT)*

pUK21-A2 (1)	GAATTCGAGC	TCCCGGGTAC	CATGGCATGC	ATCGATAGAT	CTCGAGTCTA	GACTAGAGCT
pGT	GAATTCGAGC	TCCCGGGTAC	CATGGCATGC	ATCGATAGAT	CTCGAGTCTA	GACTAGAGCT
pUK21-A2 (61)	CGCTGATCAG	CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTG	CCCCTCCCCC
pGT	CGCTGATCAG	CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT	CTGTTGTTTG	CCCCTCCCCC
pUK21-A2 (121)	GTGCCTTCCT	TGACCCTGGA	AGGTGCCACT	CCCACTGTCC	TTTCCTAATA	AAATGAGGAA
pGT	GTGCCTTCCT	TGACCCTGGA	AGGTGCCACT	CCCACTGTCC	TTTCCTAATA	AAATGAGGAA
pUK21-A2 (181)	ATTGCATCGC	ATTGTCTGAG	TAGGTGTCAT	TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC
pGT	ATTGCATCGC	ATTGTCTGAG	TAGGTGTCAT	TCTATTCTGG	GGGGTGGGGT	GGGGCAGGAC
pUK21-A2 (241)	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG	GGGAAGGCCT	CGGACTAGTG
pGT	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG	GGGAAGGCCT	CGGACTAGTG
pUK21-A2 (301)	GCGTAATCAT	GGTCATAGCT	GTTTCCTGTG	TGAAATTGTT	ATCCGCTCAC	AATTCCACAC
pGT	CCGGAATCAT	GGTCATAGCT	GTTTCCTGTG	TGAAATTGTT	ATCCGCTCAC	AATTCCACAC
pUK21-A2 (361)	AACATACGAG	CCGCGGAAGC	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC
pGT	AACATCCGGG	CCGCGGAAGC	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC
pUK21-A2 (421)	TCACATTAAT	TGCGTTGCGC	TCACTGCCCC	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC
pGT	TCACATTAAT	TCCGTTCCGC	TCACTGCCCC	CTTTCCAGTC	GGGAAACCTG	CCGTGCCAGC
pUK21-A2 (481)	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG
pGT	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGCGGTTT	CCGTATTGGC	CGCTCTTCCG
pUK21-A2 (541)	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC
pGT	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC
pUK21-A2 (601)	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT
pGT	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT
pUK21-A2 (661)	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTA AAAAGGC	CGCGTTGCTG	GCGTTTTTCC
pGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTA AAAAGGC	CGCGTTGCTG	GCGTTTTTCC
pUK21-A2 (721)	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA
pGT	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA
pUK21-A2 (781)	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC
pGT	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC
pUK21-A2 (841)	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG
pGT	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAGCGTGG
pUK21-A2 (901)	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC
pGT	CGCTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC
pUK21-A2 (961)	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC
pGT	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC
pUK21-A2 (1021)	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA
pGT	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC
pUK21-A2 (1081)	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT
pGT	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT
pUK21-A2 (1141)	ACGGCTACAC	TAGAAGAACA	GTATTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG
pGT	ACGGCTACAC	TAGAAGAACA	GTATTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG
pUK21-A2 (1201)	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT
pGT	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT
pUK21-A2 (1261)	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT
pGT	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT
pUK21-A2 (1321)	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG	AAAACACACG	TTAAGGGATT	TTGGTCATGA
pGT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG	AAAACACACG	TTAAGGGATT	TTGGTCATGA

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pUK21-A2 (1381)	GCTTGCGCCG	TCCCGTCAAG	TCAGCGTAAT	GCTCTGCCAG	TGTTACAACC	AATTAACCAA
pGT	GCTTGCGCCG	TCCCGTCAAG	TCACCGGAAT	GCTCTGCCAG	TGTTACAACC	AATTAACCAA
	-----	-----	---*---*	-----	-----	-----
pUK21-A2 (1441)	TTCTGATTAG	AAAAAATCAT	CGAGCATCAA	ATGAAACTGC	AATTTATTCA	TATCAGGATT
pGT	TTCTGATTAG	AAAAAATCAT	CCAGCATCAA	ATGAAACTGC	AATTTATTCA	TATCAGGATT
	-----	-----	-----	-----	-----	-----
pUK21-A2 (1501)	ATCAATACCA	TATTTTGGAA	AAAGCCGTTT	CTGTAATGAA	GGAGAAAAC	CACCGAGGCA
pGT	ATCAATACCA	TATTTTGGAA	AAAGCCGTTT	CTGTAATGAA	GGAGAAAAC	CACCGAGGCA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (1561)	GTTCCATAGG	ATGGCAAGAT	CCTGGTATCG	GTCTGCGATT	CCGACTCGTC	CAACATCAAT
pGT	GTTCCATAGG	ATGGCAAGAT	CCTGGTATCG	GTCTGCAATT	CCGACTCGGC	CAACATCAAT
	-----	-----	-----	-----*	-----	-----
pUK21-A2 (1621)	ACAACCTATT	AATTTCCCCT	CGTCAAAAAT	AAGGTTATCA	AGTGAGAAAT	CACCATGAGT
pGT	ACAACCTATT	AATTTCCCCT	CATCAAAAAT	AAGGTTATCA	AGTGAGAAAT	CACCATGAGT
	-----	-----	-----*	-----	-----	-----
pUK21-A2 (1681)	GACGACTGAA	TCCGGTGAGA	ATGGCAAAAAG	TTTATGCATT	TCTTTCCAGA	CTTGTTCAAC
pGT	AACACTGAA	TCCGGTGAGA	ATGGCAAAAAG	TTTATGCATT	TCTTTCCAGA	CTTGTTCAAC
	-----	-----	-----	-----	-----	-----
pUK21-A2 (1741)	AGGCCAGCCA	TTACGCTCGT	CATCAAAAATC	ACTCGCATCA	ACCAAACCGT	TATTCATTCTG
pGT	AGGCCAGCCA	TTACGCTCAT	CATCAAAAATC	GGAAGCATCA	ACCAAACCGT	TATTCATTCTG
	-----	-----*	-----	****-	-----	-----
pUK21-A2 (1801)	TGATTGCGCC	TGAGCGAGAC	GAAATACGCG	ATCGCTGTTA	AAAGGACAAT	TACAAACAGG
pGT	GGATTGAGCC	TGAGCCAGAC	GGAATACGCG	GTCGCTGTTA	AAAGGACAAT	TACAAACAGG
	-----	*-----*	*-----*	*-----*	-----	-----
pUK21-A2 (1861)	AATCGAATGC	AACCGGCGCA	GGAACACTGC	CAGCGCATCA	ACAATATTTT	GAGGTGAATC
pGT	AATGGAATGC	AACCGGCGGA	GGAACACTGC	CAGAGCATCA	ACAATATTTT	CACCTGAATC
	-----*	-----*	-----*	-----*	-----	-----
pUK21-A2 (1921)	AGGATATTCT	TCTAATACCT	GGAATGCTGT	TTTTCCGGGG	ATCGCAGTGG	TGAGTAACCA
pGT	AGGATATTCT	TCTAATACCT	GGAATGCTGT	TTTTCCGGGG	ATAGCAGTGG	TGAGTAACCA
	-----	-----	-----	-----*	-----	-----
pUK21-A2 (1981)	TGCATCATCA	GGAGTACGGA	TAAATGCTT	GATGGTCGGA	AGAGGCATAA	ATTCCGTCAG
pGT	TGCATCATCA	GGAGTACGGA	TAAATGCTT	GATGGTCGGA	AGAGGCATAA	ATTCCGTCAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2041)	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	ATCATTGGCA	ACGCTACCTT	TGCCATGTTT
pGT	CCAGTTTAGT	CTGACCATCT	CATCTGTAAC	ATCATTGGCA	ACGCTACCTT	TGCCATGTTT
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2101)	CAGAAACAAC	TCTGGCGCAT	CGGGCTTCCC	ATACAAGCGA	TAGATTGTCTG	CACCTGATTG
pGT	CAGAAACAAC	TCCGGCGCGT	CGGGCTTCCC	ATACAAGCGG	TAGATTGTAG	CACCTGATTG
	-----*	-----*	-----*	-----*	-----*	-----*
pUK21-A2 (2161)	CCCGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA	GCATCCATGT	TGGAATTTAA
pGT	CCCGACATTA	TCGCGAGCCC	ATTTATACCC	ATATAAATCA	GCATCCATGT	TGGAATTTAA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2221)	TCGCGGCCTC	GACGTTTCCC	GTTGAATATG	GCTCATAACA	CCCCTTGTAT	TACTGTTTAT
pGT	TCGCGGCCTG	GAGGTTTCCC	GTTGAATATG	GCTCATAACA	CCCCTTGTAT	TACTGTTTAT
	-----*	-----*	-----	-----	-----	-----
pUK21-A2 (2281)	GTAAGCAGAC	AGTTTATTG	TTCATGATGA	TATATTTTTA	TCTTGTGCAA	TGTAACATCA
pGT	GTAAGCAGAC	AGTTTATTG	TTCATGATGA	TATATTTTTA	TCTTGTGCAA	TGTAACATCA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2341)	GAGATTTTGA	GACACAACGT	GGCTTTCCCC	CCCCCCCCCA	TGACATTAAAC	CTATAAAAAAT
pGT	GAGATTTTGA	GACACACCGG	GGCTTTCCCC	CCCCCCCCCA	TGACATTAAAC	CTATAAAAAAT
	-----*	-----*	-----	-----	-----	-----
pUK21-A2 (2401)	AGGCGTATCA	CGAGGCCCTT	TCGTCTCGCG	CGTTTCGGTG	ATGACGGTGA	AAACCTCTGA
pGT	AGCCGTATCC	CGAGGCCCTT	CCGTCTCGCG	CGTTCCGGTG	ATGCCGGTGA	AAACCTCTGA
	-----*	-----*	-----*	-----*	-----*	-----*
pUK21-A2 (2461)	CACATGCAGC	TCCCGGAGAC	GGTCACAGCT	TGTCTGTAAG	CGGATGCCGG	GAGCAGACAA
pGT	CACATGCAGC	TCCCGGAGAC	GGTCACAGCT	TGTCTGTAAG	CGGATGCCGG	GAGCAGACAA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2521)	GCCCCGTCAGG	GCGCGTCAGC	GGGTGTTGGC	GGGTGTCGGG	GCTGGCTTAA	CTATGCGGCA
pGT	GCCCCGTCAGG	GCGCGTCAGC	GGGTGTTGGC	GGGTGTCGGG	GCTGGCTTAA	CTATGCGGCA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2581)	TCAGAGCAGA	TTGTACTGAG	AGTGCACCAT	AAAATTGTAA	ACGTTAATAT	TTTGTTAAAA
pGT	TCAGAGCAGA	TTGTACTGAG	AGTGCACCAT	AAAATTGTAA	CCGTTAATAT	TTTGTTAAAA
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pUK21-A2 (2641)	TTCGCGTTAA	ATTTTGTGTA	AATCAGCTCA	TTTTTTAACC	AATAGACCGA	AATCGGCAAA
pGT	TTCGCGTTAA	ATTTTGTGTA	AATCAGCTCA	TTTTTTAACC	AATAGACCGA	AATCGGCAAA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2701)	ATCCCTTATA	AATCAAAAGA	ATAGCCCAG	ATAGAGTTGA	GTGTTGTTCC	AGTTTGGAAC
pGT	ATCCCTTATA	AATCAAAAGA	ATAGCCCAG	ATAGAGTTGA	GTGTTGTTCC	AGTTTGGAAC
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2761)	AAGAGTCCAC	TATTAAGAA	CGTGGACTCC	AACGTCAAAG	GGCGAAAAAC	CGTCTATCAG
pGT	AAGAGTCCAC	TATTAAGAC	CGTGGACTCC	ACCGTCAAAG	GGCGAAAAAC	CGTCTATCAG
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pUK21-A2 (2821)	GGCGATGGCC	CACCCCGATT	TAGAGCTTGA	CGGGGAAAGC	CGGCGAACGT	GGCGAGAAAG
pGT	GCCGATGGCC	CACCCCGATT	TAGAGCTTGA	CGGGGAAAGC	CGGCGCGCGT	GCCGAGAAAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2881)	GAAGGGAAGA	AAGCGAAAGG	AGCGGGCGCT	AAGGCGCTGG	CAAGTGTAGC	GGTCACGCTG
pGT	GAAGGGAAGA	AACCGAAAGG	AGCGGCCGCT	AAGCCGCTGG	CAAGTGTAGC	GGTCCCCGCTG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (2941)	CGCGTAACCA	CCACACCCGC	CGCGCTTAAT	GCGCCGCTAC	AGGGCGCGTA	CTATGGTTGC
pGT	CGCGTAACCA	CCACACCCGC	CGCGCTTAAT	C CGCCGCTAC	AGGGCGCGTA	CTATGGTTGC
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3001)	TTTGACGTAT	GCGGTGTGAA	ATACCGCACA	GATGCGTAAG	GAGAAAATAC	CGCATCAGGC
pGT	TTTGCCGTAT	GCGGTGTGAA	ATACCGCACA	GATCCGTAAG	GAGAAAATAC	CGCATCAGCC
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3061)	GCCATTGCGC	ATTCAGGCTG	CGCAACTGTT	GGGAAGGGCG	ATCGGTGCGG	GCCTCTTCGC
pGT	GCCATCCGCC	ATTCAGGCTC	CGCAACTGTT	GGGAAGGCCG	ATCGGTGCGG	GCCTCTCCGC
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3121)	TATTACGCCA	GCTGGCGAAA	GGGGGATGTG	CTGCAAGGCG	ATTAAGTTGG	GTAACGCCAG
pGT	TATTCCGCCA	GCTGCCGAAA	GGGGGATGTG	CTGCAAGCCG	ATTAAGTTGG	GTACCGCCAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3181)	GGTTTTCCCA	GTCACGACGT	TGTAACACGA	CGGCCAGTGA	ATTGTAATAC	GACTCACTAT
pGT	GGTTTTCCCA	GTCACGGCGG	TGTAACCCGA	CGGCCAGTGA	ATTGTAATCC	GACTCACTAT
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3241)	AGGGCGAATT	GGGGATCGAT	CCACTAGTTC	TAGATCCGAT	GTACGGGCCA	GATATACGGG
pGT	AGGCCGAATT	GGGGACCGAT	CCACTAGTTC	TAGATCCGAT	GTACGGGCCA	GATATACGGG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3301)	TTGACATTGA	TTATTGACTA	GTTATTAATA	GTAATCAATT	ACGGGGTCAT	TAGTTCATAG
pGT	TTGACATTGA	TTATTGACTA	GTTATTAATA	GTAATCAATT	ACGGGGTCAT	TAGTTCATAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3361)	TTGACATTGA	TTATTGACTA	GTTATTAATA	GTAATCAATT	ACGGGGTCAT	TAGTTCATAG
pGT	TTGACATTGA	TTATTGACTA	GTTATTAATA	GTAATCAATT	ACGGGGTCAT	TAGTTCATAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3421)	CAACGACCCC	CGCCCATTGA	CGTCAATAAT	GACGTATGTT	CCCATAGTAA	CGCCAATAGG
pGT	CAACGACCCC	CGCCCATTGA	CGTCAATAAT	GACGTATGTT	CCCATAGTAA	CGCCAATAGG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3481)	GACTTTCCAT	TGACGTCAAT	GGGTGGAGTA	TTTACGGTAA	ACTGCCCCACT	TGGCAGTACA
pGT	GACTTTCCAT	TGACGTCAAT	GGGTGGAGTA	TTTACGGTAA	ACTGCCCCACT	TGGCAGTACA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3541)	TCAAGTGTAT	CATATGCCAA	GTACGCCCCC	TATTGACGTC	AATGACGGTA	AATGGCCCCG
pGT	TCAAGTGTAT	CATATGCCAA	GTACGCCCCC	TATTGACGTC	AATGACGGTA	AATGGCCCCG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3601)	CTGGCATTAT	GCCCAGTACA	TGACCTTATG	GGACTTTCCT	ACTTGGCAGT	ACATCTACGT
pGT	CTGGCATTAT	GCCCAGTACA	TGACCTTATG	GGACTTTCCT	ACTTGGCAGT	ACATCTACGT
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3661)	ATTAGTCATC	GCTATTACCA	TGGTGATGCG	GTTTGGCAG	TACATCAATG	GGCGTGGATA
pGT	ATTAGTCATC	GCTATTACCA	TGGTGATGCG	GTTTGGCAG	TACATCAATG	GGCGTGGATA
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pUK21-A2 (3721)	GCGGTTTGAC	TCACGGGGAT	TTCCAAGTCT	CCACCCCAT	GACGTCAATG	GGAGTTTGTT
pGT	GCGGTTTGAC	TCACGGGGAT	TTCCAAGTCT	CCACCCCAT	GACGTCAATG	GGAGTTTGTT
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3781)	TTGGCACCAA	AATCAACGGG	ACTTTCCAAA	ATGTCGTAAC	AACTCCGCCC	CATTGACGCA
pGT	TTGGCACCAA	AATCAACGGG	ACTTTCCAAA	ATGTCGTAAC	AACTCCGCCC	CATTGACGCA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3841)	AATGGGCGGT	AGGCGTGTAC	GGTGGGAGGT	CTATATAAGC	AGAGCTCTCT	GGCTAACTAG
pGT	AATGGGCGGT	AGGCGTGTAC	GGTGGGAGGT	CTATATAAGC	AGAGCTCTCT	GGCTAACTAG
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3901)	AGAACCCACT	GCTTACTGGC	TTATCGAAAT	TGCGGCCGCC	ACGGCGATAT	CGGATCCATA
pGT	AGAACCCACT	GCTTACTGGC	TTATCGAAAT	TGCGGCCGCC	ACGGCGATAT	CGGATCCATA
	-----	-----	-----	-----	-----	-----
pUK21-A2 (3961)	TGACGTCGAC	GCGTCTGCAG	AAGCTTC			
pGT	TGACGTCGAC	GCGTCTGCAG	AAGCTTC			
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Please re-write Table 6, beginning on page 64, line 1, as follows:

Table 6 *ODN used with plasmid DNA*

Backbone	ODN code number	Sequence
S-ODN	1826	TCCATGAC <u>CGTTCCTGAC</u> CGTT (SEQ ID NO:51)
	1628	GGGGTCAAC <u>CGTTGAGGGGGG</u> (SEQ ID NO:52)
	1911	TCCAGGACTTTCCTCAGGTT (SEQ ID NO:53)
	1982	TCCAGGACTTCTCTCAGGTT (SEQ ID NO:54)
	2017	CCCCCCCCCCCCCCCCCCCC (SEQ ID NO:55)
O-ODN	2061	TCCATGAC <u>CGTTCCTGAC</u> CGTT (SEQ ID NO:56)
	2001	GG <u>CGGCGGCGGCGGCGGCGG</u> (SEQ ID NO:57)
SOS-ODN	1980	TCCATGAC <u>CGTTCCTGAC</u> CGTT (SEQ ID NO:58)
	1585	GGGGTCAAC <u>CGTTGAGGGGGG</u> (SEQ ID NO:59)
	1844	TCTCCCA <u>GCGTGCGCCATAT</u> (SEQ ID NO:60)
	1972	GGGGTCTGTGCTTTTGGGGGG (SEQ ID NO:61)
	2042	TCAGGGGTGGGGGGAACCTT (SEQ ID NO:62)
	1981	GGGGTTGAC <u>GTTTTTGGGGGG</u> (SEQ ID NO:63)
	2018	TCTAG <u>CGTTTTTAGCGTTCC</u> (SEQ ID NO:64)
	2021	TCGTCGTTGTCGTTGTCGTT (SEQ ID NO:65)
	2022	TCGTCGTTTTGT <u>CGTTTTGT</u> CGTT (SEQ ID NO:66)
	2023	TCGTCGTTGTCGTTTTGT <u>CGTT</u> (SEQ ID NO:67)

SOS-ODN had two S-linkages at the 5' end, five S-linkages at the 3' end, and O-linkages in between.

Three ODN used in this study were of the same murine-specific immunostimulatory sequence in three different backbones (1826, 2061 and 1980).

All ODN were synthesized by Hybridon (Milford, MA) or Operon (Alameda, CA). ODN were ethanol precipitated and resuspended in saline prior to use alone or as an additive to the plasmid DNA solution.

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Please re-write Table 10 beginning on page 68, line 1, as follows:

Table 10

Inhibitory CpG motifs can block B cell proliferation induced by a stimulatory CpG motif

Oligonucleotide added	cpm
medium	194
1668 (TCCATGACGTTTCCTGATGCT) (SEQ ID NO:68)	34,669
1668 + 1735 (GCGTTTTTTTTTGCG) (SEQ ID NO:69)	24,452
1720 (TCCATGAGCTTCCTGATGCT) (SEQ ID NO:70)	601
1720 + 1735	1109

Splenic B cells from a DBA/2 mouse were cultured at 5×10^4 cells/100 μ l well in 96 well microtiter plates in RPMI as previously described (Krieg, *et al.*, 1995) with or without the indicated phosphorothioate modified oligonucleotides at a concentration of 60 ng/ml for 48 hr. The cells were then pulsed with ^3H thymidine, harvested, and the cpm determined by scintillation counting. The stimulatory CpG oligo 1668 was slightly but significantly inhibited by the inhibitory motifs in oligo 1735. The non CpG oligo 1720 is included as a negative control.

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Please re-write Table 11, beginning on page 69, line 1, as follows:

Table 11

Inhibitory effects of "bad" CpG motifs on the "good" CpG Oligo 1619

Notes:

The sequence of oligo 1619 is TCCATGTCCGTTCCCTGATGCT (SEQ ID NO:71)

1949 has only 1 GCG at the 3' end, which has essentially no inhibitory activity

Oligonucleotide added	IL-12 in pg/ml
medium	0
1619 alone	6
1619 + 1949 (TCCATGTCGTTCCCTGATGCG) (SEQ ID NO:72)	16
1619 + 1952 (TCCATGTCGTTCCGCGCGCG) (SEQ ID NO:73)	0
1619 + 1953 (TCCATGTCGTTCCCTGCGCGCT) (SEQ ID NO:74)	0
1619 + 1955 (GCGGCGGGCGGCGCGCGCCC) (SEQ ID NO:75)	0

Human PBMC were cultured in 96 well microtiter plates at $10^5/200\mu\text{l}$ for 24 hr in RPMI containing 10% autologous serum. Supernatants were collected at the end of the culture and tested for IL-12 by ELISA. All wells except the control (medium) contained 60 $\mu\text{g/ml}$ of the stimulatory CpG oligodeoxynucleotide 1619; stimulatory (1949) and inhibitory (all other sequences have a strong inhibitory motif) oligos were added to the indicated wells at the same concentration at the beginning of culture. All oligos have unmodified backbones.

Please re-write Table 13 beginning on page 71, line 1, as follows:

Table 13 Identification of neutralizing CpG motifs which reduce the induction of cytokine secretion by a CpG-S motif in the same ODN (*cis*-neutralization)

ODN	sequence 5'-3' ¹	ODN-induced cytokine expression ²		
		IL-6 ²	IL-12	IFN- γ
None		<5	206	898
1619	TCCATGTCGTTCCCTGATGCT (SEQ ID NO:71)	1405	3130	4628
1952GCGGCG (SEQ ID NO:73)	559	1615	2135
1953CC... (SEQ ID NO:74)	577	1854	2000

¹Dots in the sequence of ODN 1952 and 1953 indicate identity to ODN 1619; CpG dinucleotides are underlined for clarity. ODN without CpG-N or CpG-S motifs had little or no effect on cytokine production. The data shown are representative of 4 experiments.

²All cytokines are given in pg/ml; measured by ELISA on supernatants from DBA/2 spleen cells cultured in 96 well plates at 2×10^7 cells/ml for 24 hr with the indicated ODN at 30 μ g/ml. Std. dev. of the triplicate wells was <7%. None of the ODN induced significant amounts of IL-5

Please re-write Table 14 beginning on page 72, line 1, as follows:

Table 14 Inhibition of CpG-induced cytokine secretion by ODN containing CpG-N motifs

ODN	sequence 5'-3'	IL-12 secretion ¹	CpG-S-induced IL-12 secretion ²
none		268	5453
1895	GCGCGCGCGCGCGCGC (SEQ ID NO:76)	123	2719
1896	CCGCGCGCGCGCGCGC (SEQ ID NO:77)	292	2740
1955	GCGCGCGCGCGCGCGC (SEQ ID NO:75)	270	2539
2037	TCCATGCCGTTCTCTGCCGTT (SEQ ID NO:78)	423	2847

¹BALB/c spleen cells were cultured in 96 well plates at 2×10^7 cells/ml with the indicated ODN for 24 hr and then the supernatants were assayed for IL-12 by ELISA (pg/ml).

²Cells were set up the same as in ¹ except that IL-12 secretion was induced by the addition of the CpG ODN 1619 (TCCATGTCGTTCTCTGATGCT) (SEQ ID NO: 71) at 30 µg/ml. The data shown are representative of 5 experiments.